



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: John G Babish, *et al.*
Application No.: 09/885,721
Filing Date: June 20, 2001
Docket Number: 062114.0030
Title: COMPLEX MIXTURES EXHIBITING SELECTIVE
INHIBITION OF CYCLOOXYGENASE-2
Examiner: M. Meller
Art Unit: 1654

CERTIFICATE OF MAILING (37 C.F.R. § 1.8)

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to MAIL STOP AMENDMENT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date indicated below.

Date: 9/19/05


Angelo J. Mignanelli

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Sir:

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

I, Matthew L. Tripp, declare as follows:

1) I am Dr. Matthew L. Tripp, Vice President Research and Development of Metaproteomics, LLC. I have held this position since 2000.

2) I have a Doctorate degree in Bacteriology from Washington State University, as well as Masters and Bachelor degrees of Science in Biology from Western Michigan University. A copy of my Curriculum Vitae is attached as Exhibit A.

3) I am also an inventor named in several domestic and foreign patent applications including U.S. Application Nos. 08/659,807, 10/056,858, 10/352,388, 10/400,293, 10/401,283, 10/464,410, 10/464,834, 10/689,856, 10/735,526, 10/774,048, 10/789,814, 10/789,817, 10/846,948, 10/866,315, and issued foreign and domestic patents, including U.S. Patent Nos. 5,849,537; 5,783,235; 5,639,493; 5,618,572 and 5,439,699.

4) I am an expert in the art of brewing research with over ten years' experience from 1985 to 1996 at the second largest brewery in the United States, Miller Brewing Company (Milwaukee, WI) where I held the title of Manager of Process and Product Innovation.

5) I understand that in the course of the February 9, 2005 interview in the above-captioned application, Examiner M. Meller requested evidence as to the alpha acid content of beer to distinguish the compositions and methods of the invention over certain art of record. For the purposes of this Declaration, the term "alpha acids" as defined in the application denotes humulone, cohumulone, isohumulone, isoprehumulone, hulupone, adhumulone, xanthohumol A and xanthohumol B.

6) Hop constituents have a limited solubility in wort and even less so in the subsequent ferment, beer. It is my experience that beers do not contain greater than 40 ppm (40 mg/liter or 4 mg/100ml or 0.004%) of any of the alpha acids. This conclusion is based on practical first hand experience with brewing trials at the 10 liter, 40 liter, 400 barrel and 800 barrel levels. The highest bitterness units ($1\text{BU}^1=1\text{PPM}$ of any of the following:

¹ BUs are correlated to the ppm of isoalpha acids isolated from beer as analytically determined by RF-HPLC. BUs are also routinely estimated using a spectrophotometric analysis. This analysis is not accurate, however, as it also includes interference from malt components. For example, a BUDWEISER® beer with a low color (2.5 degrees SRM) and 10 ppm isoalpha acids (iso-humulone, -cohumulone, -adhumulone) as demonstrated by

isohumulone, isocohumulone, isoadhumulone) I have ever seen in extreme hop bills, and based on RF-HPLC analysis of the primary and finished beer, were in the high 30 BU (30 ppm total composite of isohumulone + isocohumulone + isoadhumulone) range. Since, humulone, cohumulone, adhumulone, lupulone, colupulone, and adlupulone for example are much less soluble than isoalpha acids, they would not come close to achieving even the 40 ppm levels.

7) *Bitterness content of selected beers.* If one searches the internet, reports of beer having BUs as high as 100 are reported (see Table 1). However, my experience in examining the dose-response of hopping bill with finished beer is that BUs as measured by RP-HPLC do not exceed about 40 BUs or 40 ppm (isohumulone, isocohumulone, isoadhumulone composite).

Table 1. International Bitterness Units scale of selected beers²

Beer	IBU [ppm Isohumulone]
BELGIAN LAMBICS™	11 - 23
BLONDE ALE™	15 - 30
KÖLSCH™	18 - 25
MÄRZEN/OKTOBERFEST™	18 - 25
ORDINARY ENGLISH BITTER™	20 - 23
PORTER™	20 - 40
BROWN ALE™	15 - 25
NORTH AMERICAN BROWN ALE™	25 - 45
BOHEMIAN-STYLE PILSENER™	30 - 45
INDIA PALE ALE™	40 or higher
Irish stout (e.g. GUINNESS™)	45 - 60
STONE RUINATION IPA™	100+

² See, Wikipedia, the free encyclopedia at <http://en.wikipedia.org/wiki/Hops>; a copy of the same is provided herewith as Attachment B. The hoppy character of beer is measured by the International Bitterness Units scale, or IBU scale. An IBU is one part per million of isohumulone. The higher number, the greater the bitterness. A light American lager might be as low as 5 on the IBU scale. At the other extreme, a barley wine can range up to 100.

8) Analysis of bitterness units (BUs) in beer. BUs are correlated to the ppm of isoalpha acids isolated from beer as analytically determined by RF-HPLC.

BUs are also routinely estimated using a spectrophotometric analysis. This analysis is not accurate, however, as it also includes interference from malt components. For example, a Budweiser beer with a low color (2.5 degrees SRM) and 10 ppm isoalpha acids (iso-humulone, -cohumulone, -adhumulone) as demonstrated by HPLC, will indicate a BU measurement of about 12-14 BU by spectrophotometric methods.

The higher the coloring of beer, the greater the interference, and a unhopped beer of 12 degrees SRM will show 4-6 BUs spectrophotometrically, but zero by HPLC. Hence the darker the beer, the poorer the spectrophotometric assay is in estimating bittering units in beer. Hence brewers use both methods routinely.

9) Maximum solubility of alpha acids in buffer pH 5.2. Malowicki and Shellhammer present information on the maximum solubility of alpha acids in pH 5.2 buffer and subsequent degradation at 70° and 120°C.³ Solubility limits for alpha acids in a pH buffered solution were determined spectrophotometrically at 328 nm. The conversion of alpha acids to iso-alpha acids was characterized in a model, laboratory scale system consisting of purified alpha acid extract in a pH buffered aqueous solution. Maximum solubility of alpha acids in a pH 5.2 buffered aqueous solution was 90 ppm. Hop isomerization kinetics examined over a broad range of temperatures showed a rate coefficient of 0.0153 sec⁻¹ for atmospheric boiling conditions. At 70°C, less than 10% of alpha acids were converted in a 90 minute boil. At 120°C, only 30 minutes were required for 90% conversion, with subsequent loss of iso-alphas to degradation products following.

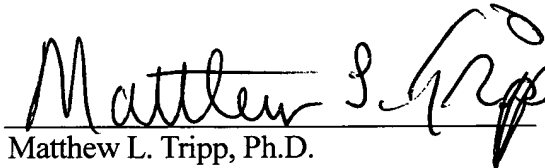
³ See, http://ift.confex.com/ift/2004/techprogram/paper_25787.htm; a copy of the same is provided herewith as Attachment C.

10) Conversion of alpha acids to isoalpha acids during boil. Glenn Tinseth⁴ describes the conversation of alpha acid to isoalpha acids during the wort boil. In the top figure, alpha acid conversion is shown to be about 25 % for a typical OG wort and in the table, maximal alpha acid conversion is about 31% with longest boil time.

11) Conclusion. Taking Malowicki and Shellhammer (*i.e.*, max alpha acid solubility is 90 ppm) and Glenn Tinseth (*i.e.*, maximum conversion is 31%) together, then $90 \text{ ppm alpha acids} \times 0.31 = 27.9 \text{ ppm isoalpha acid}$. Add an additional 4 ppm alpha acid solubility when the wort cools and you have a total 32 ppm alpha plus isoalpha acids. This figure is consistent with my experience at Miller where we never observed a concentration of isoalpha acids higher than about 40 ppm in whole hopped beer. Thus, in my opinion based on my experience, beer could contain no more than about 40 ppm or 0.04 grams/1000 ml or 0.004 grams/100 ml or 0.004% of alpha acids, a level far below the 10-95% contemplated in the patent application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: July 11, 2005


Matthew L. Tripp, Ph.D.
Vice President Research & Development
Metagenics Research Center - Suite 100
9770 44th Ave. NW
Gig Harbor, WA 98332

MIA 296896-2.068911.0030

⁴ See, <http://realbeer.com/hops/research.html>; a copy of the same is provided herewith as Attachment D.